Restriction Requirement

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-13 and 19-21, drawn to a method for large-scale production of an antibody in transformed yeast cells, vectors and host cell, classified in class 435, subclass 69.6.

II. Claims 14-18, drawn to an antibody, classified in class 530, subclass 387.1.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the antibody of Group II can be made by several different methods such as immunizing an animal with an antigen or produced in P. pastoris with different cloning steps utilizing different signal sequences and different steps to confirm the intactness of the insert, such as restriction digests, for example, in addition to the method of Group I.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art because of their recognized divergent subject matter and different classifications, restriction for examination purposes as indicated

is proper.

During a telephone conversation with Mr. James Coburn on March 1, 2000 a provisional election was made with traverse to prosecute the invention of Group I, claims 1-13 and 19-21. Affirmation of this election must be made by applicant in replying to this Office action. Claims 14-18 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Applicants correct Examiner. Examiner had a telephone conversation with the undersigned, Hana Verny, who confirms that the election with traverse was made to prosecute the Group I.

Applicants traverse Examiner's restriction requirement on the basis that the reasoning Examiner is advancing is incorrect. The antibody in claims 14-18, as presented, cannot be made by any other method then the one which is set in steps (a) through (o). However, to make this distinction absolutely clear, Applicants amended claims 14-18 to be directed to the recombinant antibody produced by the method which is identical to the method set up in claim 1 which is the subject of the Examiner's search. The claims thus being almost the same in that one set of claims is directed to a method for production of the antibody and the second to the antibody produced by the same method, it is respectfully requested that Examiner rescind his decision regarding the restriction requirement and examines both set of claims in one application. There will be no additional burden attached to searching claims 14-18.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(I).

Inventorship of the application remains the same.

Objection to the Specification

The disclosure is objected to because of the following informalities:

a. The title of the invention is not descriptive because it recites an antibody as well as the method for producing the antibody. A new title is required that is clearly indicative of the invention to which the claims are directed.

Applicants amended the title to meet Examiner's objections.

The brief description of the drawing needs to have a separate description for Figures 6A-6C.

Applicants amended the specification to meet Examiner's objections.

Objections to Drawings

The drawings are considered to be informal because they fail to comply with 37 CFR 1.84(a)(1) which requires black and white drawings using India ink or its equivalent.

a. Photographs and color drawings are acceptable only for examination purposes unless a petition filed under 37 CFR

1.84(a)(2) or (b)(1) is granted permitting their use as formal drawings. In the event applicant wishes to use the drawings currently on file as formal drawings, a petition must be filed for acceptance of the photographs or color drawings as formal drawings. Any such petition must be accompanied by the appropriate fee as set forth in 37 CFR 1.17(1), three sets of drawings or photographs, as appropriate, and, if filed under the provisions of 37 CFR 1.84(a)(2), an amendment to the first paragraph of the brief description of the drawings section of the specification which states:

> The file of this patent contain, at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Color photographs will be accepted if the conditions for accepting color drawings have been satisfied. Figure 6A-C needs to have separate views.

Applicants will file formal figures including petition to accept photographs, if such is needed, upon allowance of the application.

Rejections Under 35 USC 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-13 and 19-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-13 are indefinite for reciting incomplete method claims which do not clearly include a resolution step which reads back on the preamble of the claimed method. Merely reciting method steps, detecting the presence of the recombinant antibody by Western blot and testing the antibody for binding does not result in a method of large scale production of an antibody. The claims should conclude with a step of producing the antigen-specific intact antibody thereby producing the antibody as required by the preamble, which recites "a method for a large-scale production of antigen specific intact antibody".

Applicants disagree, however, to advance the prosecution, Applicants added step (p) directed to harvesting the recombinant antibody produced by the steps (a)-(o) of the method of invention. The steps as suggested by the Examiner would not be correct as the production of the antibody is a sequential event resulting from a strict adherence to all steps of the method. The antibody is produced by inducing the expression of recombinant antibody in steps (a)-(l) and the recombinant antibody specificity *vis-a-vis* the specific antigen is confirmed in steps m-o. After it is confirmed, the antibody is harvested in step (p).

Claims 1-13 and 20 are indefinite for reciting the term "genes' in claims 1,7,8 and 20. According to Genes IV (Levin et al,

Oxford University Press, page 810, 1990), a gene is defined as "the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding regions (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons)." From the teachings of the specification, however, the nucleic acid sequences encoding the heavy and light chain regions of the antibody do not include expression control elements that fall under the definition of a gene. Accordingly, the claims are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants disagree, however, to meet Examiner's rejections, Applicants canceled the term "gene" or "genes" from the claims.

Claims 1-13 and 19-21 are indefinite because they contain the abbreviation "P. pastoris" and "PCR" in claims 1, 2, 7, 10, and 11. Full terminology should be in the first instance of the claims followed by the abbreviation in parentheses. Dependent claims may then use the abbreviation. Abbreviations render the claim indefinite because the same abbreviation may represent more than one element or concept.

Applicants made appropriate corrections in all independent claims.

Claims 1-13 are indefinite for reciting "sequence" in claims 1(e), (g), (k), and 13. The term "sequence" refers to information describing the nucleic acid or amino acid sequence. Information is not a chemical structure, therefore, it is not clear how

"sequences" can be linked to nucleic acid molecules. Replacing this term with polynucleotide, DNA, RNA or polypeptide, as appropriate, would be sufficient to obviate this rejection.

Applicants introduced language suggested by the Examiner.

Claim 1 recites the limitation of expression cassettes in the claim. There is insufficient antecedent basis for this limitation in the claim. Is "said expression cassettes" to mean those recited in claims 1(a) or 1(c) or 1(d)?

Applicants amended claim 1 to specifically identify which expression cassettes are meant.

Claims 1-13 are indefinite for reciting "preparing and transforming *P. pastoris* with BglIl, NotI, Sad, SalI or Stu 1-linearized recombinant plasmid" in claim 1 for the exact meaning of the phrase is not clear. It is not clear if the phrase is meant to mean that all of the recited restriction sites are to be used of if the claim is meant to recite a Markush group. In addition it is unclear what "prepared" is to encompass. Does it mean to digest the plasmid, set the vector out on the table, or some other steps? As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants amended claims to clarify step (g) and make to it more definite.

Claims 1-13 are indefinite for reciting "the AOX1 promoter fused to a Saccharomyces cerevisiae-mating factor signal sequence" in claim 1(e) for the exact meaning of the phrase is not clear. It is unclear how the expression cassettes, which are DNA, are to be

fused to a α-mating factor signal sequence, which is a protein. The claim should recite that the DNA encodes the signal sequence. As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants amended step (e) as suggested.

Claims 2-13 are indefinite for reciting "cDNA in tandem....flanked by a P. pastoris signal sequence" in claim 2 for the exact meaning is not clear. It is not clear how DNA can be flanked by a protein signal sequence. The claim should recite flanked by DNA encoding the signal sequence. As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants amended claim 2 as suggested.

The term "large-scale production" in claims 1 and 21 is a relative term which renders the claim indefinite. The term "large-scale production" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Applicants disagree, however, to advance the prosecution, Applicants canceled the term "large scale" from the claims.

Claims 6 and 7 are indefinite for reciting the term "pPICZαLH" because other laboratories/inventors may use the same laboratory designation to refer to different plasmids.

Claims 6 and 7 are amended.

Claim 19 is indefinite for reciting "cDNA copy of immunoglobulin light and heavy chain" for the exact meaning of the phrase is not clear. It is not clear how the cDNA copy can be a protein. Is the phrase meant to mean cDNA copy of DNA encoding immunoglobulin light and heavy chain proteins? As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants amended claim 19 to meet Examiner's rejection.

Claim 20 is indefinite for reciting "system" for the exact meaning of the term is unclear. Does the term mean a kit, a method, a vector, or something else? As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants disagree but upon reconsideration, Applicants amended claims to be directed to a vector rather than to a system transformed with human, mouse or humanized mouse immunoglobulin.

Claims 1-13, 20 and 21 are indefinite for reciting "intact antibody" or "intact antibodies" in claims 1, 20, and 21 for the exact meaning of the phrase is not clear. Does the phrase mean an intact antigen binding site, an entire antibody, etc? As written, it is impossible to determine the metes and bounds of the claimed invention.

Applicants disagree but changed the language in the claims 1-13, 20 and 21.

It is believed that all rejections are overcome with amendments. Examiner is respectfully requested to withdraw the rejections under 35 U.S.C. 112, second paragraph in view of the above amendments.

Claim Rejections Under 35 USC §101

35 U.S.C. 101 reads as follows:

"Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereto, may obtain a patent therefor, subject to the conditions and requirements of this title."

Claim 20 is rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. The claim recites "system" and it is not known what applicant is claiming. Is the "system" a process, machine, manufacture, or composition of matter?

Applicants disagree with Examiner' argument but to expedite the prosecution, Applicants amended Claim 20 to be directed to a vector.

Claim Rejections Under 35 USC §102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of

application for patent in the United States.

Claims 19-21 are rejected under 35 U.S.C. 102(b) as being anticipated by Eldin et al (J. of Immunological Methods, 201:67-75, 2/14/97) and as evidenced by the Invitrogen 1997 catalog (published 1197, Yeast expression pages 14-17).

The Examiner provide basis for this rejection as follows.

The claims recite a recombinant P. pastoris vector containing dual expression cassettes, each carrying a cDNA copy of immunoglobulin light and heavy chain and P. pastoris transformed with DNA encoding an antibody. For this rejection, the term "system" in claim 20 is being interpreted as an expression vector and the P. pastoris yeast transformed for expression of heterologous proteins in P. pastoris and the phrases "intact antibody" or "intact antibodies" are being interpreted as an antibody or antigen binding fragment that comprises an intact antigen binding site.

Eldin et al teach a recombinant P. pastoris yeast expression vector (pPIC9) for expression of an antibody, which contains an intact antigen binding site, and the P. pastoris yeast transformed with the expression vector comprising cDNA encoding an immunoglobulin light and heavy chain (see page 68-69). As evidenced by the Invitrogen 1997 catalog the pPIC9 vector is a designed to contain multiple copies of the gene of interest (see page 17) thus meeting the Claim Rejections Under 35 USC §103.

Applicants disagree. In order to be anticipated, the invention must be the same. Applicants respectfully suggest that

the inventions are not the same and that the current invention is clearly distinguished from the Eldin referenced.

The current invention is directed to the intact antibodies, not to the two single chain Fv fragment as disclosed in Eldin. By producing the two expression cassettes, subcloning them into two vectors and by combining the two vectors into one plasmid for expression of both light and heavy chains of the antibody, *Pichia pastoris* is transformed to secrete the complete "intact" monoclonal antibody.

"Intact" antibody in this application does not mean, as Examiner interprets it "the antibody which contains an intact binding site" but means an entire antibody expressed according to the invention which contains two intact inserts, that is, two exact replicas of the two expression cassettes corresponding to the whole antibody consisting of the light and heavy chain and not fragments as described by Elgin.

Reference to the intact antibodies in the application is made on page 1, lines 30-34 where the intact antibodies are described as complex multimeric proteins of which production typically, and before this invention, require post translational modifications in a functionally assembled form. Additionally, on page 25, lines 11-16, Applicants again state that the intact antibody is a complex multimeric protein.

"Multimeric" means an active aggregate resulting from the interaction of polypeptide monomers (Glossary of Genetics and Crytogenetics, 4th Ed., Springer-Verlag, New York (1976).

By definition, then, the intact antibodies of the invention are not antibody single chain Fv (sFv) fragments of Eldin (see Abstract, 1st and 2nd lines). In the introduction, Eldin states that "antibody single chain Fv (sFv) **fragments** are potentially more effective than whole antibodies...". Eldin further continues that sFv fragments contain heavy and light chain variable (V_H AND v_L) regions connected by a small, flexible peptide and, as such, are about **a fifth the size of intact IgG.**

Eldin does describe the secretion of two murine sFv fragments, however, these fragments do not form one antibody. They are simply two different fragments from two different antibodies, see last paragraph in 1st column bridging to the 2nd column, page 68. The method is also different. Cloning (§2.1) for example, includes modification of sFv construct to include C-terminal cysteine codon to facilitate linking the expressed sFv fragment to other proteins. Selection and screening (§2.2) are also different. For example, AOX1 gene was replaced with 3AlfsFveys construct, p. 69, 1st col., last paragraph, and His transformants were selected for 3 days at 30°C. A screening for clones secreting proteins was by growing His transformant per construct for **2 days** at 30°C and incubated for another **2 days**. The time course of protein secretion was followed by expression (§2.3) of clones for **2 days** and incubation for **4 days** and another incubation for **4 days**. Eldin needed 17 days of various incubations, shaking, growing in the medium, etc., **to get to the expression of the fragment clones.**

Contrary to this laborious and lengthy method of Eldin which at the end produces only the fragment of the antibody, the current method produces the whole (intact) recombinant antibody all in from 12-120 hours, with the highest levels of production in 72-108 hours (3-4.5 days) (Spec. P. 25, lines 3-10).

Eldin clearly does not anticipate the current claims 19-21 as it does not result in the same product, i.e., the vector containing dual expression cassettes encoding the whole intact antibody v. single chain Fv fragment and such product is not derived from the same method and not in the same time. Time saving feature is extremely important for meaningful production of antibody in large quantities. The amount of the produced antibody is also much higher for the current method in time relation. Whereas Eldin produced up to 250 mg/l of the fragment in more than 17 days (Abstract), the current invention produces 100-360 mg/l (10-36 mg/l^{-1}) in 12 hours to 4.5 days. That is approximately four times as much of the whole antibody than Eldin produces.

Eldin does not anticipate the current invention. The rejection under 35 USC 102(b) is overcome and should be withdrawn. It is respectfully requested that Examiner do so.

Rejection under 35 U.S.C. 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102

of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham V. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 (a) are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35

U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Horwitz et al (Proc. Natl. Acad. Sci. USA 85:8678-8682, 1988) and further in view of Cregg et al (Developments in Industrial Microbiology 29:33-41, 1988) and The Invitrogen 1997 Catalog (published 1/97, Yeast expression pages 14-17 and Master Catalog Amendment Notice for pPICZ vectors from 4/15/96) and Sambrook et al (Molecular Cloning, A Laboratory Manual Second Edition pages 1.85, 12.16-12.20, and 13.42-13.44, 1989).

The claims encompass a method for large-scale production of an antibody by isolating DNA for the light and heavy chain and assembling the DNA into a P. pastoris yeast expression vector and transforming S. cerevisiae with the plasmid under control of the AOX1 promoter fused to the alpha-mating factor, transforming P. pastoris with a plasmid comprising the AOX1 antibody DNA, selectively growing and screening for expression, sequencing the DNA for integrity, detecting the presence of the antibody by Western blot, and testing the antibody for antigen binding. Further embodiments are a method wherein the antibody DNA are assembled into an expression cassette comprising P. pastoris promoter AOX1 at the 5'-terminus and a P. pastoris transcription termination sequence at the 3'-terminus and wherein the expression vector is pPICZa, the AOX1-antibody DNA is inserted by homologous recombination replacement, the selection is on medium containing zeocin, the screening is by colony-immunoblotting, restriction analysis, and the DNA inserts are confirmed by nucleotide sequence

analysis. For this rejection the phrase "intact antibody" in claim 1 is being interpreted as an antibody or antigen binding fragment that comprises an intact antigen binding site.

Here, Applicants wish to reiterate that the intact antibody does not mean fragment or antigen binding fragment. It literally means the whole intact antibody.

Examiner argues that Horwitz et al teach a method for the production of an antibody in S. cerevisiae yeast cells with the vectors comprising cDNA encoding for an antibody, a promoter and transcription terminator, and signal sequence (see abstract and page 8679 and figure 2). The vector was constructed with molecular biology methods and the recombinants were screened using selective conditions (see page 8680). The detection of the recombinant antibody was done by Western blot (see page 8680) and antigen-antibody binding was performed (see page 8680). Horwitz et al does not teach a recombinant host P. pastoris transformed with a vector for expression, the AOX1-P promoter, the pPICZa vector, replacement of the yeast chromosomal AOX1 with the AOX1-antibody DNA by homologous recombination, or selection on zeocin media or screening by colony-immunoblotting, restriction analysis or nucleotide sequence analysis. These deficiencies are made up for in the teachings of Cregg et al, the Invitrogen 1997 Catalog, and Sambrook et al.

Cregg et al teach production of foreign proteins in Pichia pastoris with the promoter AOX1.

Sambrook teach basic molecular biology methods and screening colonies by colony immunoblotting, restriction analysis, and nucleotide sequence analysis.

The Invitrogen 1997 Catalog teach the pPICZα vector which uses the zeocin resistant polynucleotide for selection in P. pastoris and comprises the inducible AOX1 promoter, a poly cloning site comprising EcoRI, BsmBI, BglII, and BamHI, the α-factor signal sequence, and the vector is designed for antibody expression.

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a method for production of an antibody in *P.pastoris* comprising the claimed steps with the vectors and methods of selection, screening, detection, and binding analysis in view of Horwitz et al, Cregg et al, Sambrook et al, and the 1997 Invitrogen Catalog in order to produce antibodies in P. pastoris.

One of ordinary skill in the art would have been motivated to produce the claimed method because Horwitz et al teach recombinant production of proteins, specifically, an antibody in S. cerevisiae in general with selection, screening, and purification and testing antigen binding. In addition, one of ordinary skill in the art would have been motivated to produce the claimed method in P. pastoris because Cregg et al teach production of heterologous proteins in P. pastoris overcomes the problems associated with producing commercially useful levels of proteins in S. cerevisiae (see page 33, introduction) and the P. pastoris is ideally suited for the production of many heterologous proteins due to the fact

that (1) a detailed understanding of the growth characteristics of the organism in high-density fermentors is known, (2) the ability to place foreign DNA into the genome in a precisely controlled manner, and (3) promoters are tightly regulated and efficiently transcribed to produce proteins at high levels. (See page 40). In addition, one of ordinary skill in the art would have been motivated to produce the claimed method because the Invitrogen Catalog teach a Pichia expression vector called pPICZα which is based on homologous recombination comprising several restriction sites for cloning of recombinant proteins, a promoter (AOX1), termination sequences, selectable markers (zeocin), and α-factor secretion signal for expression in P. pastoris of antibodies (see pages 14-15 and 18). Moreover, one of ordinary skill in the art would have been motivated to construct vectors for cloning and methods of screening of transformed colonies for expression cassettes because Sambrook et al teach basic molecular biology methods for cloning and screening of transformed colonies and in view of the teachings of Sambrook one skilled in the art would also reasonably conclude that when constructing recombinant vectors one would naturally analyze the DNA sequence for integrity and intactness and perform screening methods for obtaining the desired colonies. In addition in view of the teachings of Horwitz et al one of ordinary skill in the art would know to use the methods of Western blot for detection of the expressed protein and test the antibody for antigen binding.

Moreover, one of ordinary skill in the art would have had a reasonable expectation of success in producing a method for production of an antibody in P. pastoris because Horwitz et al teach the antibodies produced in yeast were secreted and functional by binding the target antigen (see abstract). In addition, one of ordinary skill in the art would have had a reasonable expectation of success in producing a method for production of an antibody in P. pastoris because Cregg et al teach the result of the engineered yeast is a yeast that is "easily scaled up from shake-flask to large-volume, high-density cultures with little change in the kinetics of reasonable expectation of success in producing a method for production of an antibody in P. pastoris because the Invitrogen Catalog teach that the expression vector and P. pastoris makes "an ideal tool for laboratory research as well as industrial applications" (see page 14).

Therefore, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Applicants disagree. Examiner offers only general observations without understanding the intricacies of the problems which the invention solved. Obvious advantages of the current method over prior art methods make this invention unobvious. Moreover, to derive the *prima facie* obviousness, Examiner has to combine, in a rather complicated way, no less than four (4) publications available from 1988-1997 to all "one of ordinary skill in the art" who then had an ample opportunity to come up with such

an "obvious" method as Examiner would have us believe. Applicants suggest that Examiner uses hindsight which makes everything obvious to him. However, to expedite the prosecution, claims were extensively amended.

Brief Description of the Invention

The method of the invention produces an entire intact antibody, that is it produces an exact replica of the whole antigen-specific antibody raised against the specific antigen by constructing two expression cassettes corresponding to and each comprising the light and/or heavy chains DNAs of the target antibody. The cassettes are flanked by a signal sequence preceded by a yeast promoter and by the yeast termination DNA sequence. Plasmid arrangement is protective of the whole DNA sequence.

In the current invention, the *P. pastoris* is transformed with recombinant plasmid, such as pPICZαLH and zeocin-resistant transformants are isolated.

A schematic representation of expression cassettes which are unique to this invention is shown in Figure 1 of the specification. The recombinant plasmid, pPICZαLH, contains a bacterial origin of replication (COIEI), Zeocin-resistance gene (Zeo^R) for selection of both *E. coli* and yeast transformants, and the expression cassettes of antibody light and heavy chain DNAs exemplarized by the anti-dioxin antibody DNAs.

The final construct is assembled by replacement-ligation of the *MluI-Bam*HI fragment (for dioxin) of the recombinant plasmid construct containing the light chain expression cassette, and the

*MluI-Bam*HI fragment from the heavy chain expression cassette construct.

As seen in Figure 1, the expression cassettes of the light-chain (L-chain) and heavy-chain (H-chain) DNAs are each fused to the yeast α-factor signal sequence (SS), under the control of the yeast promoter (AOX1-P) at 5'-terminal. A yeast transcription termination sequence (TT) marks the 3' end of each expression cassette. The expression cassettes are thus special entities expressed as such and are protective of the antibody intactness. They are not expressing, and do not permit expression of, other entities such as individual DNA fragments.

Results obtained with using the expression cassettes as illustrated in the specification for dioxin, confirm the integrity of expression cassettes and the junction sequences by DNA sequence analysis.

PCR analysis of *P. pastoris* transformants prepared according to the invention using primers specific for the antibody genes or for the AOXI 5' and 3' termini shows intact full-length light- and heavy-chain gene expression cassettes integrated in genomic DNA of the transformants. Control transformants harboring vector alone yield no amplification products in PCR with Ab gene primers. DNA sequence analysis of the PCR products from recombinants is used to confirm that the primary structure of the target sequences is preserved.

The antibody production by recombinant *P. pastoris* clone is easily induced. Induction of recombinant antibody expression is

typically performed as follows. A *P. pastoris* transformant is cultured for two days at 30°C. The yeast cells are collected by centrifugation and transferred to the induction medium. Beginning on the second day of growth, methanol is added daily to induce the AOX1 promoter-driven production of recombinant antibody.

The method involves making a nitrocellulose membrane-replica of recombinants on an agar plate with induction medium and probing it with AP-goat anti-mouse IgG.

Transcripts detected in the Northern blot are specific and correspond to the sizes expected for both light- and heavy chains.

The intactness of the inserts and the accuracy of the junction sequences are easily confirmed using PCR procedure by nucleotide sequence analysis.

Antibody levels in cell lysates is approximately 10% of the total product, as assessed by Western blotting, and are consistently lower than those found in culture medium. This demonstrates that a major portion, approximately 90% of the antibody produced by recombinant *P. pastoris*, according to the invention, is secreted into the supernatant in an amount from about 10-36 mg/l in about 12 hours to 4.5 days. These results show that *P. pastoris* secretes intact antibody very efficiently. These results are unexpected, surprising and very advantageous for rapid preparation of large quantities of a very specific antibody.

Studies performed to determine the kinetics of monoclonal antibody have shown that synthesis and secretion of antibody are optimal between 72 and 108 hours. Using the current method, anti-

hapten antibody was detectable in culture medium between 12 hours and 120 hours of induction, with highest levels of about 10 to 36 mg/l detected between 72 and 108 hours.

Although these levels are lower than those reported for other recombinant proteins or for antibody fragments (200 mg/l), they are the highest and set the highest range ever obtained for any intact antibody, which is a more complex multimeric glycoprotein, than the molecules previously reported. These levels can be further augmented by using fermentation approaches.

This invention thus provides a method of general utility for production of large quantities of any antigen-specific antibody using modified yeast organism. Using the method of invention, the large quantity of antigen specific and defined monoclonal antibody is produced without the necessity of immunizing and recovering and purifying antibodies and/or other lengthy procedures. The method is practical, economical, easy, safe and fast and in about three days, the monoclonal antibody is produced by the transformed yeast if the vector and expression vehicles for transformation are available or are prepared according to the invention.

The above described findings demonstrate the suitability of *P. pastoris* expression system for both small and large-scale production of functional, antigen-specific intact antibodies. The recombinant antibodies produced by the method of the invention are useful, for example, for immunodiagnostic and immunotherapeutic purposes. Since recombinant proteins produced in *P. pastoris* lack terminal α-1,3-glycan linkages responsible for hyper-

immunogenicity, the antibodies produced in *P. pastoris* are particularly suitable for therapeutic applications.

Analysis of References

Horwitz reference describes secretion of functional antibody and the Fab fragment from yeast cells. To construct the functional antibody, cDNA copies of the chimeric light chain and heavy chain genes were inserted into separate vectors for each chain (see Figure 2A). The vector pING1441 comprising the complete 2-μm plasmid, the chimeric light chain gene V and C_K regions fused to the PGK promoter P invertase signal sequence S and PGK transcription termination and polyadenvlylation signal T. The vector pING1442 for heavy chain contains the yeast origin replication (oriY) and a cis-acting stabilization sequence Rep3 from yeast 2-μm plasmid, the chimeric heavy chain gene V-region and C region domains C_{H1}, C_{H2} and C_{H3} fused to PGK promoter, invertase signal sequence and PGK transcription termination and polyadenlyation signals.

These plasmids are entirely different entities from each other and unconnected to each other.

Moreover, a cloning strategy of Horwitz consists of fusing cDNA coding for the mouse V regions at the immunoglobulin joining J region to cDNA coding for human IgG1 C regions.

While the method of Horwitz would seem to produce antibody containing both heavy and light chains, the production is extremely inefficient as only about 100 ng (nanograms) per ml is produced in 3 days which corresponds to about 100 micrograms per liter,

compared to the current method producing about 100-360 times more, about 10-36 milligrams per liter in the same time.

Moreover, as Examiner admits, Horwitz does not teach a recombinant host *P. pastoris* transformed with a vector for expression, the AOX1-P promoter, the pPICZa vector, replacement of the yeast chromosomal AOX1 with the AOX1-antibody DNA by homologous recombination, or selection on zeocin media or screening by colony-immunoblotting, restriction analysis or nucleotide sequence analysis. These deficiencies, however, according to the Examiner, are made up for in the teachings of Cregg et al, the Invitrogen 1997 Catalog, and Sambrook et al.

Applicants do not think so. As described in the current specification, production of antibodies is much more complicated that production of recombinant proteins as described by Cregg. Antibody, which is a complex multimeric glycoprotein present challenging problems.

The fact that although both Horwitz and Cregg references were available to persons skilled in the art since 1988 and although clearly there was a need to produce complex multimeric proteins in large quantities surpassing those produced in animals, in a shorter amount of time than needed for production of antibodies by animals, there is no prior art which would do so. That alone shows that the invention is not obvious from Horwitz in view of Cregg.

Examiner would like to overcome this clear lack of nonobviousness by adding reference Invitrogen which makes the *Pichia pastoris* pPICZα vector available for cloning and selection.

But again, until late 1998, no person skilled in the art came up with such, according to the Examiner, "obvious" solution of the truly serious problem.

No person skilled in the art would have expected from either Horwitz, who produced very small quantities (about 100x less) of recombinant antibodies or from Cregg, who produced noncomplex simple proteins, that by introducing the method of generating expression cassettes containing both heavy and light chains and expressing them using *Pichia pastoris*, one could produce a large quantity of complex multimeric proteins, i.e., antibodies in about 3-4 days.

Molecular cloning reference added by the Examiner does not add anything. Anybody who knows anything about molecular biology knows of and uses Sambrook Laboratory Manual. Does that mean that all inventions which use any method described in Sambrook are obvious. Quite clearly not. Besides, this reference was also known and available since 1989 so why did no one (skilled or nonskilled) in the art come up with the current method?

The answer is clear. No one came up with the method of the invention because it is not obvious, because it is surprising and unexpected to produce 10-36 mg of fully assembled functional entire (intact) antibody in 12-120 hours, that is, in the latest 4-5 days. The method is simple, fast, reproducible and nonobvious. T h e rejection should be withdrawn.

Claims 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Horwitz et al further in view of The 1997

Invitrogen Catalog.

The claims have been described supra. For this rejection, the term "system" in claim 20 is being interpreted as an expression vector and the P. pastoris yeast transformed for expression of heterologous proteins in P. pastoris.

Horwitz et al has been discussed supra. Horwitz et al does not teach an expression vector for production of antibodies in P. pastoris and transformants of P. pastoris. This deficiency is made up in the teachings of the 1997 Invitrogen Catalog.

The Invitrogen catalog has been described supra. The Invitrogen catalog also teaches the pAO815 vector. This vector was designed to generate multiple copies of the gene of interest in a single vector (See page 17).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a recombinant P. pastoris vector containing dual expression cassettes, each carrying a cDNA copy of immunoglobulin light and heavy chain and P. pastoris transformed with cDNA encoding an antibody in view of Horwitz et al and the 1997 Invitrogen Catalog.

One of ordinary skill in the art would have been motivated to produce the claimed expression vector and P. pastoris transformed with expression cassettes for expression of an antibody because the Invitrogen Catalog teach a Pichia expression vector called pAO815 which "is specially designed to generate multiple copies of the gene of interest in a single vector" and "increasing the number of

copies of the gene of interest in a recombinant Pichia strain may increase protein expression levels" (see page 17). Thus, it would have been obvious to one of skill in the art to combine the DNA encoding for an antibody as taught by Horwitz et al in the expression vectors as taught by the invitrogen catalog in order to produce high levels of expression of the antibody in P. pastoris.

Moreover, one of ordinary skill in the art would have had a reasonable expectation of success in producing an expression vector and P. pastoris transformed with expression cassettes for expression of an antibody because Horwitz et al teach the antibodies produced in yeast were secreted and functional and bound the target antigen (see abstract). Moreover, one of ordinary skill in the art would have had a reasonable expectation of success in producing an expression vector and P. pastoris transformed with expression cassettes for expression of an antibody because the Invitrogen Catalog teach that improved expression vectors and P. pastoris makes fermentors as small as I liter and as large as 10,000 liters (see page 14).

Therefore, Examiner suggests, the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references.

Applicants disagree with rejection of claims 19-21 as being obvious from Horwitz in combination with Invitrogen Catalog.

The question, as in the prior rejection, is why no one made such combination. Here, Examiner claims that it would be obvious to produce a recombinant *P. pastoris* vector containing dual

expression cassettes, each carrying a copy of cDNA encoding antibody light and heavy chain.

The reason why it was not done is because Horwitz does not produce or describe expression cassettes, first, and second, the yield of Horwitz production is so low that no one interested in improving a yield would have been interested to do so, particularly when it takes 3-5 days to grow the transformants producing only 100 nanograms/ml (see p. 8680, 1st. Col., 1st full paragraph) and when Horwitz clearly states that to prepare sufficient quantities of yeast-derived antibody, the transformant needs to be grown in a 10-liter fermentor for another 58 hours, adding yet another 2 days to the production (see 2nd full paragraph).

In any case, although both references were there for all to see, nobody apparently did see, make such connection or thought it would be obvious to derive the vector pPICZα for large scale production of intact antibodies.

Claims 19-21 are not obvious and the rejection should be withdrawn.

SUMMARY

In summary, claims 1-21 are extensively amended to take into consideration Examiner's rejections. Applicants provided arguments showing that the invention is not anticipated or obvious from the prior art. It is believed that with this amendment, all claims are in condition for allowance. Notice of Allowance is respectfully requested.

Respectfully submitted,

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